

Claims

1. A method for purifying a target protein from a protein solution containing the target protein by using liquid chromatography, wherein

the liquid chromatography comprises:

a first step of introducing the protein solution into a column filled with a packing agent and causing the packing agent to hold the target protein; and

a second step of eluting the target protein by using an eluent containing a hydroxy-cholate.

2. The method for purifying protein according to Claim 1, wherein the target protein contains an electron transfer protein.

3. The method for purifying protein according to Claim 2, wherein the target protein is provided by a glucose dehydrogenase containing a protein having glucose dehydrogenation activity.

4. The method for purifying protein according to Claim 1, wherein the packing agent is provided by an ion-exchange gel.

5. The method for purifying protein according to Claim 4,
wherein the ion-exchange gel contains a quaternary ammonium
group as an ion-exchange group.
- 5 6. The method for purifying protein according to Claim 3,
wherein the electron transfer protein has a molecule weight
of approximately 43 kDa in SDS-gel electrophoresis under
a reducing environment,
the protein which has glucose dehydrogenation activity
10 having a molecule weight of approximately 60 kDa in SDS-gel
electrophoresis under a reducing environment.
7. The method for purifying protein according to Claim 1,
wherein the hydroxy-cholate comprises a cholate.
- 15 8. The method for purifying protein according to Claim 1,
wherein the hydroxy-cholate in the eluent is maintained at
a constant concentration in the elution of the target protein
from the packing agent.
- 20 9. The method for purifying protein according to Claim 8,
wherein the concentration of the hydroxy-cholate in the
eluent is selected from a range of 0.5 through 2.5 wt%.
- 25 10. The method for purifying protein according to Claim 3,
wherein the glucose dehydrogenase is produced by a
microorganism belonging to the genus Burkholderia.

11. The method for purifying protein according to Claim 10,
wherein the microorganism belonging to the genus *Burkholderia*
is provided by *Burkholderia cepacia* KS1 strain (FERM
BP-7306).

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12. The method for purifying protein according to Claim 3,
wherein the glucose dehydrogenase is produced by a
transformant,

the transformant being produced by engineering a host
10 microorganism with a DNA from a microorganism belonging to
the genus *Burkholderia* for coding the electron transfer
protein and the protein active against glucose.

13. The method for purifying protein according to Claim 12,
15 wherein the host microorganism is provided by *Pseudomonas*
putida.

14. The method for purifying protein according to Claim 12,
wherein the host microorganism is provided by *E. coli*
20 bacterium.

15. A method for purifying glucose dehydrogenase using a
combination of hydrophobic chromatography and anion exchange
chromatography, wherein
25 the hydrophobic chromatography includes: a step of
causing a stationary phase to hold the glucose dehydrogenase;
a step of eluting unnecessary proteins; and a step of eluting

the glucose dehydrogenase by using an eluent containing a hydroxy-cholate,

the anion exchange chromatography including: a step of causing a stationary phase to hold the glucose dehydrogenase; and a step of eluting the glucose dehydrogenase by using an eluent containing a hydroxy-cholate.

16. The method for purifying glucose dehydrogenase according to Claim 15, wherein concentration of the hydroxy-cholate in the eluent is varied with time in the hydrophobic chromatography,

concentration of the hydroxy-cholate in the eluent being kept at a constant level in the elution of the glucose dehydrogenase in the anion exchange chromatography.

17. The method for purifying glucose dehydrogenase according to Claim 16, wherein the anion exchange chromatography is carried out after the hydrophobic chromatography.

18. The method for purifying glucose dehydrogenase according to Claim 15, wherein the glucose dehydrogenase is produced by a microorganism belonging to the genus Burkholderia.

19. The method for purifying glucose dehydrogenase according to Claim 18, wherein the microorganism belonging to the genus Burkholderia is provided by Burkholderia cepacia KS1 strain (FERM BP-7306).

20. The method for purifying glucose dehydrogenase according to Claim 15, wherein the glucose dehydrogenase is produced by a transformant,

5 the transformant being produced by engineering a host microorganism with a DNA from a microorganism belonging to the genus Burkholderia for coding the glucose dehydrogenase.

21. The method for purifying glucose dehydrogenase according to Claim 20, wherein the host microbe is provided by Pseudomonas putida.

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22. The method for purifying glucose dehydrogenase according to Claim 20, wherein the host microbe is provided by E. coli bacterium.

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23. The method for purifying glucose dehydrogenase according to Claim 15, wherein the anion exchange chromatography uses an ion-exchange gel containing a quaternary ammonium group as an ion-exchange group,

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the hydroxy-cholate being provided by a cholate.